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PREFERENCE FOR ODOR-TASTE MIXTURES IS DEPENDENT ON
PREVIOUS EXPERIENCE

By

Kelsey Allison McQueen
B.S., University of Louisville, 2017
M.S., University of Louisville, 2019

A Thesis
Submitted to the Faculty of the
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In Anatomical Sciences and Neurobiology

School of Medicine
Department of Anatomical Sciences and Neurobiology
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Louisville, Kentucky

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A Thesis Approved on

April 25, 2019

By the following Thesis Committee:

Thesis Director: Dr. Chad Samuelson

Second Committee Member: Dr. Robert Lundy

Third Committee Member: Dr. Peter Kaskan

DEDICATION

This thesis is dedicated to my parents

Mr. Charles McQueen

and

Mrs. Pamela McQueen

who have given me invaluable educational opportunities.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Chad Samuelson, for his guidance and patience. I would also like to thank the other committee members, Dr. Robert Lundy and Dr. Peter Kaskan, for their comments and assistance over the past two years. I would also like to express my thanks to Dr. Jennifer Brueckner-Collins, who inspired me to never give up on my dream. In addition, many thanks to Christian Dahlgren who helped me through the hard times. Finally, I would like to thank all of my friends that kept me sane along the way.

ABSTRACT

PREFERENCE FOR ODOR-TASTE MIXTURES IS DEPENDENT ON PREVIOUS EXPERIENCE

Kelsey A. McQueen

April 25, 2019

The perception of flavor occurs when a tastant is simultaneously detected and associated with an odorant (i.e., odor-taste mixture). Sampling an odor and taste together results in a congruent odor-taste mixture. Mixing an odor and taste from different congruent odor-taste pairs results in incongruent odor-taste mixtures. Creation of a flavor percept requires sampling a novel chemosensory stimulus; however, mammals exhibit robust neophobic behavior when presented with new chemosensory stimuli. To determine preference for novel odorants and experienced odor-taste mixtures, we employ a two-bottle brief-access preference task where two chemosensory stimuli are presented simultaneously. We found that rats show a preference for water over a novel odor until the odor is paired with a pleasant taste. Additionally, rats prefer an odor-taste mixture containing the odor previously paired with a pleasant taste, regardless of odor-taste congruence. Finally, we show that rats prefer a novel odor to an experienced-unpleasant odor, but prefer an experienced-pleasant odor.

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INTRODUCTION

The senses of smell and taste are used to discriminate edible foods from potentially hazardous ones. When we eat, our senses of smell (olfaction) and taste (gustation) are activated simultaneously and interact with one another (Verhagen and Engelen, 2006; Small and Green, 2012). This multisensory interaction generates long-lasting odor-taste associations, referred to as flavors (Sclafani, 2001; Small and Green, 2012). The concept of flavor, however, has been misrepresented through common terminology. For example, the phrase “tastes like strawberry” is technically incorrect, since a strawberry contains both taste and odor molecules. A *flavor* is the perception that occurs from the pairing of an odor with a taste (odor-taste association). Instead of “tastes like strawberry”, one should say “strawberry flavor” or “strawberries have a sweet taste”.

There are only five *taste* qualities: sweet, salty, sour, bitter, and umami (savory). The study of taste centers on understanding how chemicals that stimulate taste receptor cells in taste buds lead to the perception of these qualities. Taste signals are transmitted to the brain via three cranial nerves: facial (CN VII), glossopharyngeal (CN IX), and the vagus nerve (CN X) (see review

Carleton et al., 2010). The chorda tympani branch of CN VII carries taste information from the anterior two-thirds of the tongue, CN IX carries taste information from the posterior one-third of the tongue, and CN X carries some taste information from the back of the throat. Information from these cranial nerves, together with somatosensory information from the trigeminal nerve, is conveyed through peripheral sensory neurons to the taste portion of the nucleus of the solitary tract (NST) (Spector and Travers, 2005). In most mammals, gustatory signals are next transmitted to neurons in the parabrachial nucleus (PBN), then to the parvocellular portion of the ventroposteromedial nucleus of the thalamus (VPMpc) (Tokita et al., 2009). In primates, projections from the gustatory portion of the NST bypass the PBN and sending information directly to VPMpc (Beckstead et al., 1980). Next, gustatory signals from VPMpc project to the primary cortical area for taste, gustatory cortex (GC) (Shi and Cassell, 1998). In turn, GC then projects to higher-order cortical, thalamic, and limbic regions involved in processing the sensory and affective properties of flavors (Samuelsen et al., 2012; Jezzi et al., 2013; Maier et al., 2015). This proposed 'flavor network' includes regions integral to the sensory processing of odors (Small, 2012; Small and Green, 2012).

The sense of smell (olfaction) depends upon volatile chemical odors (i.e., odorants) activating olfactory sensory neurons (OSNs) in the nasal epithelium. There are two functionally distinct routes for odors to reach the nasal epithelium: orthonasal and retronasal olfaction (Heilmann and Hummel, 2004; Small et al., 2005; Gautam and Verhagen, 2012b; Gautam et al., 2014). Orthonasal olfaction

occurs when odors are sampled via the nostrils (e.g. sniffing a flower). Retronasal olfaction occurs when odors from the mouth travel back through the oropharynx during exhalation (Masaoka et al., 2010; Gautam and Verhagen, 2012a) and is a necessary component for the perception of flavors (Lim and Johnson, 2011; Prescott, 2012). Olfactory signals are transmitted via the olfactory nerve (CN I), which is made up of the axons of the olfactory receptor neurons embedded in the olfactory epithelium (Arzi and Sobel, 2011). The OSNs project to the olfactory bulb in the frontal cortex, where they synapse with mitral and tufted cells. Axons of mitral and tufted cells emerge from the main olfactory bulb (MOB) to form the lateral olfactory tract (Hadley et al., 2004). Unlike all other sensory systems, the olfactory pathway does not project to thalamus before reaching cortex. Efferent fibers from the MOB project to multiple cortical olfactory areas (i.e., olfactory cortex), the largest of which is piriform cortex (Neville and Haberly, 2004; Wilson and Sullivan, 2011). Similarly to the taste pathway, piriform cortex then projects to higher-order cortical, thalamic, and limbic regions involved in processing the sensory and affective properties of flavors (Zald and Pardo, 1997; Courtiol and Wilson, 2014; Maier et al., 2015).

While the gustatory and olfactory pathways are separate entities, the interaction between the two is crucial for the perception of flavor (Schul et al., 1996). Interactions between gustatory cortex, piriform cortex, and higher-order cortical areas, such as orbitofrontal cortex (OFC) are thought to give rise to the perception of flavor (Rolls and Baylis, 1994; Small et al., 2004). Another area with direct connections from both chemosensory systems is the amygdala. It forms

reciprocal connections with gustatory cortex, piriform cortex, PBN, and NST (Krettek and Price, 1977; Haberly and Price, 1978; Shi and Cassell, 1998). These amygdalar connections are believed to contribute to the hesitation to try new foods, the avoidance of those that smell novel, and rejection of those that have been associated with illness (Bielavska and Roldan, 1996; Zald and Pardo, 1997; Dardou et al., 2007; Lin et al., 2009a).

When a food is novel, mammals tend to avoid it or eat less of it, a behavior termed neophobia (Barnett, 1958). Since rodents are unable to vomit, they must be extremely cautious of what they ingest. When presented with a new food, wild rats will avoid it for a long period of time (Rzóska, 1953). Eventually, they will sample it and, after a few days, will either accept it or reject it (Barnett and Spencer, 1953). When presented with a choice between familiar and novel stimuli, rats will always show a strong preference for the familiar one (Barnett, 1956). For example, when given a single bottle containing a novel odor dissolved in water (odorized-water), rats initially avoid drinking it; however, since the novel stimulus is the only available one to consume, rats will sample it more on subsequent days (Miller et al., 1986; Lin et al., 2009b; Fredericksen et al., 2019). Although experience with a chemosensory stimulus reduces neophobia, it is unclear whether the increased sampling over time represents a change in the value of the odor. In other words, do rats drink more of the chemosensory stimulus overtime because they begin to “like” it?

When eating a novel food is a pleasant experience, it becomes hedonically positive and will be preferred in the future (Sclafani, 2001). A taste with a positive

hedonic value, when paired with a neutral odor, causes a shift in the hedonic value of the odor (Fanselow and Birk, 1982). Experiments have shown that rats prefer an odor previously associated with a pleasant taste (e.g., saccharin or NaCl) and avoid an odor that has been associated with an aversive taste (e.g., quinine) (Fanselow and Birk, 1982; Schul et al., 1996; Sakai and Yamamoto, 2001; Sakai and Imada, 2003). According to White and Prescott 2007, smelling the odor of a previously sampled odor-taste mixture leads to the expectation of the paired taste due to the implicit association between odor-taste pairings (White and Prescott, 2007). For example, when an odor that is perceived as “sweet” is added to a sucrose solution, the mixture is rated sweeter than sucrose alone (Stevenson et al., 1995). These learned odor-tastes associations convey that previously paired odor-taste mixtures belong together (congruent), while mixing an odor and a taste from different congruent odor-taste pairs is a violation of the learned associations (incongruent) (Schifferstein and Verlegh, 1996; Amsellem and Ohla, 2016). For example, vanilla extract is often added to sugary desserts, resulting in the smell of vanilla being described as sweet and pleasant. Therefore, an odor-taste mixture of vanilla and sucrose would commonly be perceived as congruent, whereas a mixture of vanilla and citric acid (sour) would be incongruent. In fact, an odor previously paired with sucrose suppresses the sourness of a citric acid solution (Stevenson, 1999). Furthermore, it has been shown that chemoresponsive areas of the brain (i.e., anterior cingulate cortex, orbitofrontal cortex, and insular cortex) are activated by congruent odor-taste mixtures, but not by incongruent mixtures (Small et al., 2004).

It is unknown how experience with odor-taste mixtures impacts preferences for chemosensory stimuli. To test this gap in knowledge, I tested how prior experience influences preferences for orally-consumed odor-containing stimuli (i.e., odorized-water and odor-taste mixtures). Specifically, I hypothesized that: 1) water will be preferred to odorized-water; however, experiencing that odor mixed with a pleasant taste (i.e., sucrose) will change the odor preference, 2) after experiencing two odor-taste mixtures, one pleasant and one unpleasant, odor-taste mixtures containing the pleasant odor will be preferred regardless of odor-taste congruence. Furthermore, I tested the hypothesis that 3) an odor previously paired with an unpleasant taste will be preferred to a novel odor.

These hypotheses were tested using a two-bottle brief-access task, which presented the rats with a choice between two bottles containing liquid stimuli. Where a single-bottle task is a measure of the motivation to consume a single stimulus, a two-bottle brief-access task measures which stimulus is preferred during a limited period of time. In this task, the rats could choose which bottles to lick and were able to switch between stimuli. The rat's preference for chemosensory stimuli can be inferred by measuring which stimulus has been sampled the most.

In summary, preference for a chemosensory stimulus requires repeated experience with novel chemosensory stimuli to overcome robust neophobic behavior. Hesitance to sample new foods affects future food choices and preference. Once neophobia subsides, mammals are able to form a preference based on both the odor and taste (the human concept of flavor) of a stimulus.

Since mammals exhibit neophobia when presented with novel odors and also avoid an odor previously paired with an unpleasant taste, it is unclear which has a greater influence on preference. I will explore whether these preferences are influenced more by the hedonic value of a paired odor, or by the congruency of the odor-taste mixture.

MATERIALS AND METHODS

Subjects. All experimental procedures were performed in accordance with university, state, and federal regulations regarding research animals and were approved by the University of Louisville Institutional Animal Care and Use Committee. Thirteen (Experiment 1, n=5; Experiment 2, n=8) naïve, 3-month-old female Long-Evans rats (200-300 g; Charles Rivers) were maintained on a 12/12-hr light-dark cycle with *ad libitum* access to food and water unless otherwise specified.

Two-bottle brief-access preference task. To assess preference for odorized-water as well as odor-taste mixtures, I utilized a custom-built computer-controlled two-bottle brief-access apparatus. All of the two-bottle brief-access preference task experiments followed this general protocol. Water-regulated rats (see below) were allowed 5 minutes of habituation in the test chamber before the preference task was initiated via custom-written LabVIEW scripts (National Instruments Austin, TX). A trial began with the simultaneous opening of two port doors: each port allowing access to a bottle containing a stimulus. All bottle pairings were counterbalanced so that both bottles were presented at each port five times (ten trials total). Once the doors opened, rats had 15s to contact either bottle to initiate a trial; if no contact was made, the doors closed, and the program continued to the next trial. However, if either bottle was contacted during the initial 15s, the doors remained open for an additional 15s. Individual licks were recorded via a grounded

circuit. After each trial, the doors closed, and a 15s inter-trial interval began where a new pair of stimuli was moved to the ports.

Experiment 1: Preference for novel odor. All rats (n=5) were placed on a water regulation cycle. Rats were allowed access to distilled water for 1h/day in their home cage and trained to drink water in the test chamber. After three days of training, data was recorded for the preference task (as above). A trial began with the simultaneous opening of two port doors: one port allowed access to a sipper tube containing water, and the other allowed access to an identical sipper tube containing either water (days 1-3), 0.01% isoamyl acetate (IA) (days 4-6 and day 8), or a mixture of 0.01% isoamyl acetate-0.1 M sucrose (day 7) (Figure 1). Bottles



Figure 1. Schematic outline of the two-bottle brief-access task: preference for novel odor. After 3 days of habituation to the test apparatus, water-regulated rats were given the choice between a bottle containing water and one containing water (days 1-3; black bar), 0.01% isoamyl acetate (days 4-6 and day 8; magenta bar) or a bottle containing a mixture of 0.01% isoamyl acetate-0.1 M sucrose (day 7, yellow bar). All two-bottle choices were counterbalanced such that chemosensory stimuli were presented five times at each port.

were counterbalanced so that each pairing was presented five times at each port (ten trials total). The data are presented as the mean numbers of licks per 15s trial and as preference ratios. The mean number of licks is calculated as the average number of licks of each bottle containing the same stimulus across trial days. The preference ratios are calculated as $(B1 - B2) / (B1 + B2)$, where B1 is the total number of licks for counterbalanced water and B2 is the total number of licks for the counterbalanced stimulus. A positive preference score indicates a preference

for water while a negative score indicates a preference for the chemosensory stimulus.

Experiment 2A: Congruency vs. Hedonic Value. All rats (n=8) were allowed access to distilled (di) water for 4h/day in their home cage after undergoing trials in the rig during experiment days. Three training days were carried out, where rats were placed in the test chamber for 5 min (training day 1), 10 min (training day 2), or 15 min (training day 3) and allowed to habituate without door operation. During these training days, rats were given overnight, home-cage access to congruent odor-taste pairings of 0.01% isoamyl acetate-0.2 M sucrose (IA-S) and 0.01% benzaldehyde-0.3 M citric acid (B-CA). After three days of odor-taste mixture experience, rats began the congruency vs. hedonic value task (Figure 2). A trial began with the simultaneous opening of two port doors: choices between congruent odor-taste mixtures IA-S and B-CA (days 1,3,4,6), experienced odors

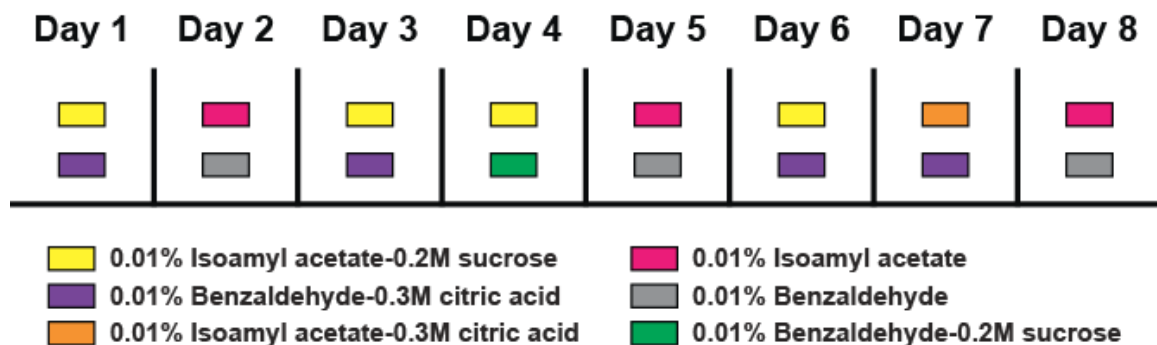


Figure 2. Schematic outline of the two-bottle brief-access task: congruency vs. hedonic value. Water-regulated rats were given 3 training days to habituate to the test apparatus. After each training day, rats were given home cage access to two odor-taste mixtures: 0.01% isoamyl acetate-0.2 M sucrose and 0.01% benzaldehyde-0.3 M citric acid. After this training period, rats were given the choice between bottles containing 0.01% isoamyl acetate-0.2 M sucrose (yellow bar) and 0.01% benzaldehyde-0.3 M citric acid (purple bar) (days 1,3,6), 0.01% isoamyl acetate (magenta bar) and 0.01% benzaldehyde (grey bar) (days 2,5,8), 0.01% isoamyl acetate-0.2 M sucrose (yellow bar) and 0.01% benzaldehyde-0.2 M sucrose (green bar) (day 4), and 0.01% isoamyl acetate-0.3 M citric-acid (orange bar) and 0.01% benzaldehyde-0.2 M citric acid (purple bar) (day 7). All two-bottle choices were counterbalanced such that chemosensory stimuli were presented five times at each port.

IA and B (days 2,5,8), incongruent 0.01% benzaldehyde-0.2 M sucrose (B-S) and congruent IA-S (day 4), and incongruent 0.01% isoamyl acetate-0.3 M citric acid (IA-CA) and congruent B-CA (day 7). Bottles were counterbalanced so that each pairing was presented five times at each port (ten trials total). The data are presented as the mean numbers of licks per 15s trial and as preference scores, calculated as $(B1 - B2) / (B1 + B2)$, where B1 is the total number of licks for counterbalanced isoamyl acetate-containing stimuli and B2 is the total number of licks for benzaldehyde-containing stimulus. A positive preference score indicates a preference for isoamyl acetate while a negative score indicates a preference for benzaldehyde.

Experiment 2B: Unpleasant-experienced vs. neophobia. The same group of rats for experiment 2A (n=8) were used for Experiment 2B. All rats were allowed access to distilled water for 4h/day in their home cage and retrained to drink water in the test chamber. After one day of odor-taste mixture training in the rig with IA-S vs. B-CA, rats began the task (Figure 3). Trials began with the simultaneous opening of two port doors: choices between congruent odor-taste mixtures IA-S and B-CA (days 1 and 3), experienced odors IA and B (day 2), unpleasant-experienced odor B and novel 0.01% methyl valerate (MV) (day 4), and pleasant-experienced odor IA and MV (day 5). Bottles were counterbalanced so that each pairing was presented five times at each port (ten trials total). The data are presented as the mean numbers of licks per 15s trial and as preference ratios, calculated as above. A positive preference score indicates a preference for isoamyl

acetate, IA while a negative score indicates a preference for either benzaldehyde or methyl valerate, depending on the trial day.

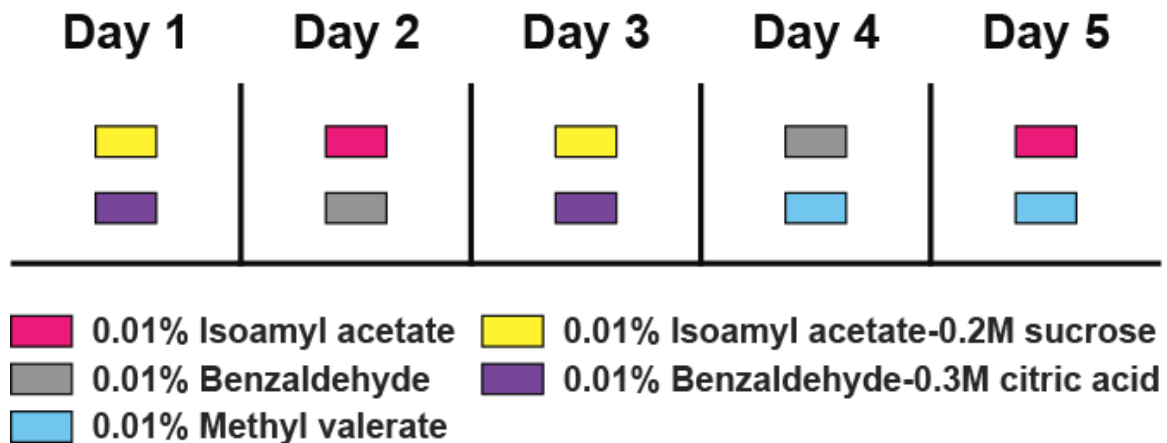


Figure 3. Schematic outline of the two-bottle brief-access task: experienced-unpleasant vs. neophobia. The same group of rats from Experiment 2A were given a single day of odor-taste mixture training (0.01% isoamyl acetate-0.2 M sucrose vs. 0.01% benzaldehyde-0.3 M citric acid) in the test apparatus. After this single training day, rats were given the choice between bottles containing 0.01% isoamyl acetate-0.2 M sucrose (yellow bar) and 0.01% benzaldehyde-0.3 M citric acid (purple bar) (days 1 and 3), 0.01% isoamyl acetate (magenta bar) and 0.01% benzaldehyde (grey bar) (day 2), 0.01% benzaldehyde (grey bar) and 0.01% methyl valerate (cyan bar) (day 4), and 0.01% isoamyl acetate (magenta bar) and 0.01% methyl valerate (cyan bar) (day 5). All two-bottle choices were counterbalanced such that chemosensory stimuli were presented five times at each port.

Statistical analysis. All statistical analyses were performed using GraphPad Prism (GraphPad Software: San Diego, CA). For Experiment 1, comparisons of the mean number of licks for water and stimuli across days was determined using a two-way repeated-measures ANOVA. Comparisons between preference ratios were made utilizing a one-way repeated-measures ANOVA. For Experiments 2A and 2B, a two-way repeated-measures ANOVA was used to determine if the mean number of licks for odor-taste mixtures or odors changed over days. Significant differences in mean number of licks between pairs of chemosensory stimuli were determined with two-tailed paired *t*-tests. Comparisons between the mean number of total licks across days were made using a one-way repeated-measures ANOVA. Comparisons between preference ratios were made using a one-way repeated-

measures ANOVA. For Experiment 2B, one rat's scores on experimental day 5 were lost due to a computer malfunction, therefore comparisons between preference ratios were made using a mixed-effects model analysis. *Post hoc* analyses included Holm-Sidak tests for multiple comparisons to correct for familywise errors.

RESULTS

Experiment 1: Preference for Novel Odor. Rodents show strong neophobic behavior when presented with a novel chemosensory stimulus (Barnett, 1958; Miller et al., 1986; Lin et al., 2009b). However, after being exposed to the stimulus for an extended period of time, sampling will increase and the rat will either form a preference or an aversion to the odor-taste pairing (Best et al., 1978). To examine how experience influences the preference for an orally-consumed odor, I employed a two-bottle brief-access preference task (Fredericksen et al., 2019). In this task, rats were given the choice to drink from two simultaneously presented bottles within a limited period of time. The results of a two-way repeated-measures ANOVA comparing the mean number of licks for water and chemosensory stimuli across trial days revealed a significant main effect of stimulus [$F(1,8) = 15.15$, $P = 0.0046$], no difference across trial days [$F(7,56) = 0.7821$, $P = 0.6050$], and a significant interaction between stimulus and trail day [$F(7,56) = 4.34$, $P = 0.0005$] (Figure 4A). A *post hoc* analysis comparing the mean number of licks between the two-bottle pairs for each trial day showed a significant preference for water over isoamyl acetate on day 4 ($t_{(64)} = 3.44$, $P < 0.01$), day 5 ($t_{(64)} = 4.96$, $P < 0.01$), and day 6 ($t_{(64)} = 2.731$, $P < 0.05$). When given the choice between water and an isoamyl acetate-sucrose mixture, rats sampled them similarly (day 7: $t_{(64)} = 0.1798$, $P > 0.05$). After experiencing the isoamyl acetate-sucrose odor-taste mixture, there

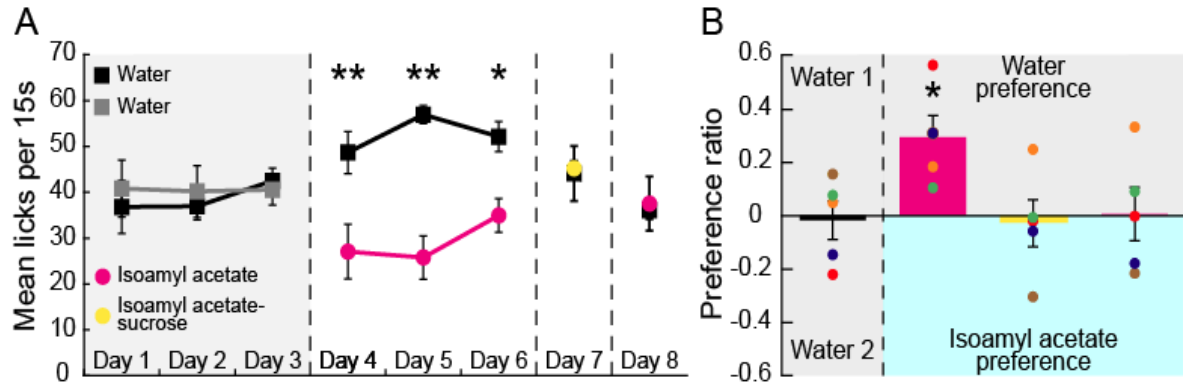


Figure 4. Experience with an odor-taste mixture changes odor preference. (A) Mean licks per 15 s (\pm SEM) during the two-bottle brief-access task. Rats given the choice between 0.01% isoamyl acetate-odorized water and water, significantly prefer to drink water (days 4–6). There was no preference when given the choice between 0.01% isoamyl acetate mixed with 0.1 M sucrose (odor-taste) and water (day 7). The day after an experience with the odor-taste mixture (day 8), the preference for water over odorized water was eliminated. (B) Preference ratios (\pm SEMs) for each two-bottle choice. Preference ratios were averaged for days 1–3 (water vs. water) and days 4–6 (water vs. isoamyl acetate). Prior to experience with the odor-taste mixture, the preference for water over isoamyl acetate (left magenta bar) was significantly greater than the preference ratios for water/water (black bar), water/isoamyl acetate-sucrose (yellow bar), and water/isoamyl acetate after odor-taste experience (right magenta bar). Colored circles represent an individual rats' average preference ratio for each two-bottle choice. * $P < 0.05$ ** $P < 0.01$ (From Fredericksen, McQueen, and Samuelsen, 2019).

was no significant difference in the number of licks for water over isoamyl acetate odorized-water (observed on days 4-7) (day 8: $t_{(64)} = 0.2291$, $P > 0.05$).

Preference ratios were calculated to determine whether preferences changed with experience (Figure 4B). These ratios represent which of the two bottles, the water or the chemosensory stimulus, was sampled more during each two-bottle task; a positive preference ratio indicates a preference for water, while a negative ratio indicates a preference for the stimulus. As there was no significant difference across days for water versus water (days 1-3) or water versus isoamyl acetate (days 4-6), preference ratios were averaged. The results of a one-way repeated-measures ANOVA showed a significant difference between preference ratios [$F(3,16) = 3.323$, $P = 0.0465$]. A *post hoc* analysis showed that the preference ratio for water/isoamyl acetate (prior to sampling an odor-taste mixture)

was significantly different from all of the other two-bottle choices (water/water: $t_{(16)} = 2.597$, $P < 0.05$; water/isoamyl acetate-sucrose: $t_{(16)} = 2.692$, $P < 0.05$; water/isoamyl acetate: $t_{(16)} = 2.413$, $P < 0.05$). The results of the two-bottle brief-access task show that the initial avoidance of a novel orally consumed odor continues when the rat is given a choice, but pairing the odor with a pleasant taste stimulus eliminates the preference for water over odorized-water.

Experiment 2A: Congruence vs. Hedonic Value. Sampling a novel odor and a taste together associates the odor with the hedonic value of the taste, making the odor-taste pair congruent (Schifferstein and Verlegh, 1996; Gautam and Verhagen, 2010). Mixing a congruent odor with a taste has been shown to increase the intensity of the taste (Dalton et al., 2000; Diamond et al., 2005; White and Prescott, 2007). Additionally, the results of Experiment 1 showed that after an unpaired odor is mixed with a pleasant taste, preference shifted towards the odorized-water (Figure 4). To test whether the odor-taste mixture preference is due to the congruency of the odor-taste pairing or the hedonic value of the experienced odor, I employed a two-bottle brief-access odor preference task. Rats were given experience with two odor-taste mixtures, one pleasant (0.01% isoamyl acetate-0.2 M sucrose) and one unpleasant (0.01% benzaldehyde-0.3 M citric acid). To confirm that experience modulated preferences for odor-taste mixtures and odorized-water, I first ran a two-way repeated-measures ANOVA comparing the mean number of licks for isoamyl acetate-sucrose and benzaldehyde-citric acid across trial days. This revealed a significant main effect of stimulus [$F(1,14) = 321.2$, $P < 0.001$], no difference across trial days [$F(2, 28) = 0.3726$, $P = 0.6598$],

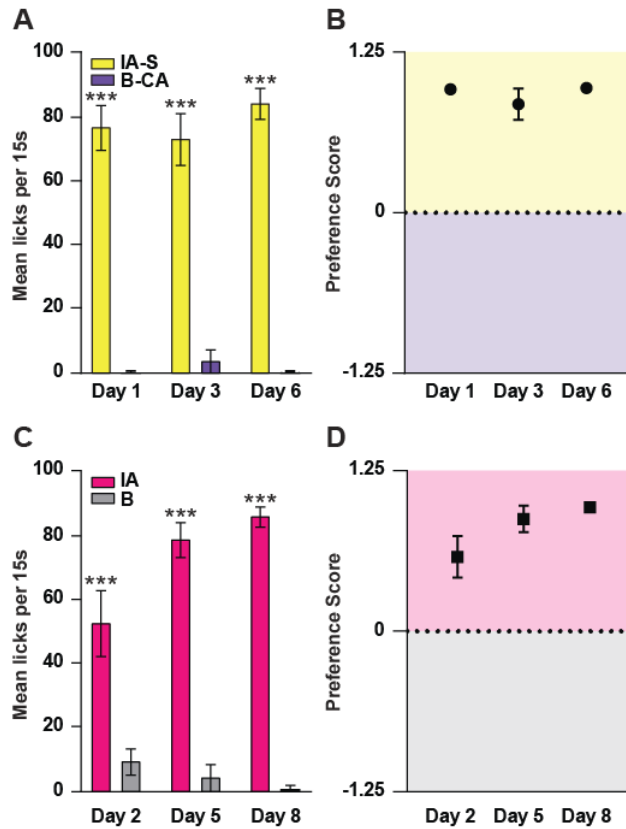


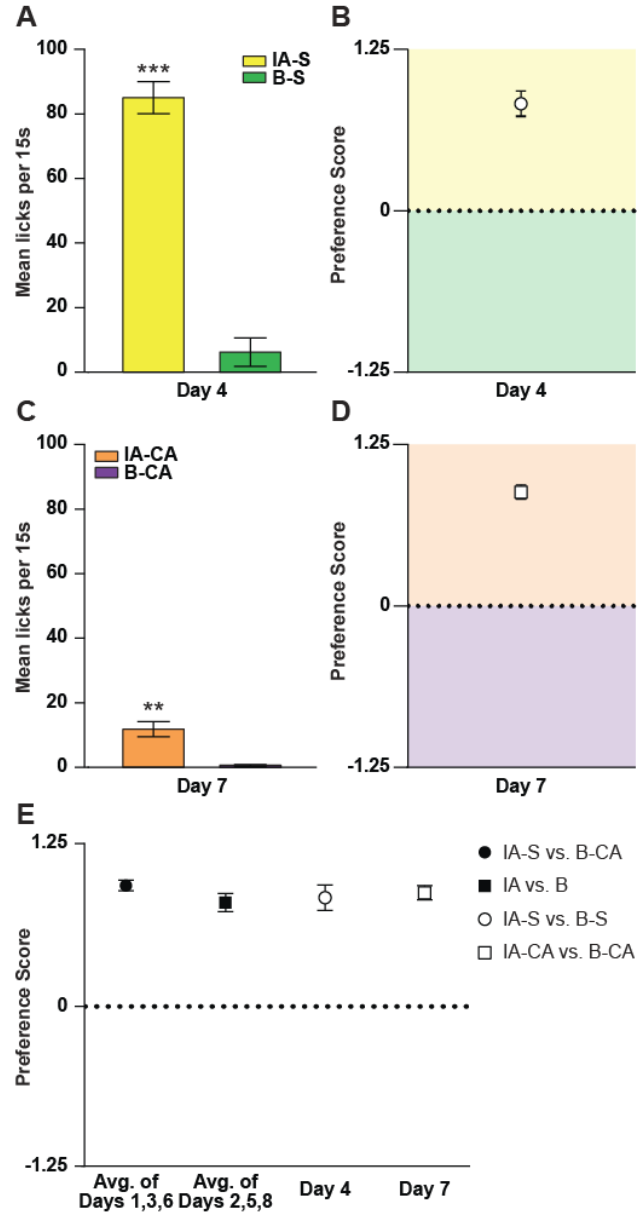
Figure 5. Preference for isoamyl acetate does not change over time. **(A)** Mean licks per 15 s (\pm SEM) during the two-bottle brief-access task across trial days 1, 3, and 6. Rats given the choice between 0.01% isoamyl acetate-0.2 M sucrose (IA-S) and 0.01% benzaldehyde-0.3 M citric acid (B-CA) on days 1, 3, and 6, prefer to drink more IA-S. **(B)** Preference scores (\pm SEM) for 0.01% isoamyl acetate-0.2 M sucrose (IA-S) and 0.01% benzaldehyde-0.3 M citric acid (B-CA) across trial days 1, 3, and 6. A positive preference score indicates a preference for IA-S while a negative preference score indicates a preference for B-CA. Preference scores did not significantly differ across days (one-way ANOVA; $P = 0.3907$). **(C)** Mean licks per 15 s (\pm SEM) during the two-bottle brief-access task across trial days 2, 5, and 8. Rats given the choice between 0.01% isoamyl acetate (IA) and 0.01% benzaldehyde (B) on days 2, 5, and 8, prefer to drink more IA. **(D)** Preference scores (\pm SEM) for 0.01% isoamyl acetate (IA) and 0.01% benzaldehyde (B) across trial days 2, 5, and 8. A positive preference score indicates a preference for IA while a negative preference score indicates a preference for B. Preference scores did not significantly differ across days (one-way ANOVA; $P = 0.0568$). *** $P < 0.001$.

and no significant interaction between stimulus and day [$F(2, 28) = 1.142$, $P = 0.3338$] (Figure 5A). A *post hoc* analysis comparing the mean number of licks between the two-bottle pairs for each trial day showed a significant preference for isoamyl acetate-sucrose over benzaldehyde-citric acid (day 1: $t_{(42)} = 10.83$, $P < 0.001$; day 3: $t_{(42)} = 9.81$, $P < 0.001$; day 6: $t_{(42)} = 11.89$, $P < 0.001$). Additionally, the results of a two-way repeated-measures ANOVA comparing the mean number of licks for isoamyl acetate and benzaldehyde across trial days revealed a significant main effect of stimulus [$F(1, 14) = 180.3$, $P < 0.001$], no difference across trial days [$F(2, 28) = 3.337$, $P = 0.0672$], and a significant interaction between stimulus and day [$F(2, 28) = 9.053$, $P = 0.0009$]

(Figure 5C). A *post hoc* analysis comparing the mean number of licks between the two-bottle pairs for each trial day showed a significant preference for isoamyl acetate over benzaldehyde (day 2: $t_{(42)} = 3.897$, $P < 0.01$; day 5: $t_{(42)} = 10.77$, $P < 0.001$; day 8: $t_{(42)} = 25.77$, $P < 0.001$).

To determine how an odor influences the preference for odor-taste mixtures, the rats were given two separate two-bottle preference tasks (Figure 6). The first task was to determine whether rats prefer a pleasant tasting congruent (isoamyl acetate-sucrose) or a pleasant tasting incongruent (benzaldehyde-

Figure 6. Preference for chemosensory stimuli is driven by hedonic value. **(A)** Mean licks per 15 s (\pm SEM) during the two-bottle brief-access task on day 4. Rats given the choice between 0.01% benzaldehyde-0.2 M sucrose (B-S) and 0.01% isoamyl acetate-0.2 M sucrose (IA-S), significantly prefer IA-S. **(B)** Preference score (\pm SEM) for 0.01% isoamyl acetate-0.2 M sucrose (IA-S) and 0.01% benzaldehyde-0.2 M sucrose (B-S) for trial day 4. A positive preference score indicates a preference for IA-S while a negative preference score indicates a preference for B-S **(C)** Mean licks per 15 s (\pm SEM) during the two-bottle brief access on day 7. Rats given the choice between 0.01% isoamyl acetate-0.3 M citric acid (IA-CA) and 0.01% benzaldehyde-0.3 M citric acid (B-CA), drink significantly more IA-CA. **(D)** Preference score (\pm SEM) for 0.01% isoamyl acetate-0.3 M citric acid (IA-CA) and 0.01% benzaldehyde-0.3 M citric acid (B-CA) for trial day 7. A positive preference score indicates a preference for IA-CA while a negative preference score indicates a preference for B-CA. **(E)** Preference scores (\pm SEM) for the four two-bottle brief-access choices: IA-S vs. B-CA (average preference score of days 1, 3, 6), IA vs. B (average preference score of days 2, 5, 8), IA-S vs. B-S (day 4), and IA-CA vs. B-CA (day 7). A positive preference score indicates a preference for IA-containing stimuli while a negative preference score indicates a preference for B-containing stimuli. Preference scores did not differ significantly between two-bottle brief-access choices (one-way ANOVA; $P = 0.4108$). *** $P < 0.001$.



sucrose) odor-taste mixture. The second task was to determine whether rats prefer an unpleasant tasting congruent (benzaldehyde-citric acid) or an unpleasant tasting incongruent (isoamyl acetate-citric acid) odor-taste mixture. The results of a two-tailed paired t-test found that rats sampled the bottle containing isoamyl acetate-sucrose significantly more than a bottle containing benzaldehyde-sucrose (IA-S: 86.49 ± 5.01 vs. B-S: 6.77 ± 4.47 ; $t_{(7)} = 8.464$, $P < 0.001$) (Figure 6A). Furthermore, rats sampled the bottle containing

isoamyl acetate-citric acid significantly more than a bottle containing benzaldehyde-citric acid (IA-CA: 12.44 ± 2.44 vs. B-CA: 0.512 ± 0.35 ; $t_{(7)} = 4.739$, $P = 0.0021$) (Figure 6C).

Preference ratios were calculated as in Experiment 1 to determine whether preferences for a stimulus changed across trial days. A positive preference ratio indicated a preference for a stimulus containing isoamyl acetate, while a negative preference ratio indicated a preference for a stimulus containing benzaldehyde. As preference scores did not significantly differ across days for isoamyl acetate-sucrose/benzaldehyde-citric acid trials (days 1,3,6) (Figure 5B) [one-way repeated measures ANOVA; $F(2, 14) = 1.006$, $P = 0.3907$] or the isoamyl acetate/benzaldehyde trials (days 2,5,8) (Figure 5D) [one-way repeated measures ANOVA; $F(2, 14) = 3.546$, $P = 0.0568$] preference ratios were averaged. Results of a one-way repeated-measures ANOVA comparing preference scores between

all two-bottle choices found no significant differences in preference ratios across stimulus type [$F(3, 21) = 1.003$, $P = 0.4108$] (Figure 6E).

However, the results of a one-way repeated measures ANOVA revealed that the mean

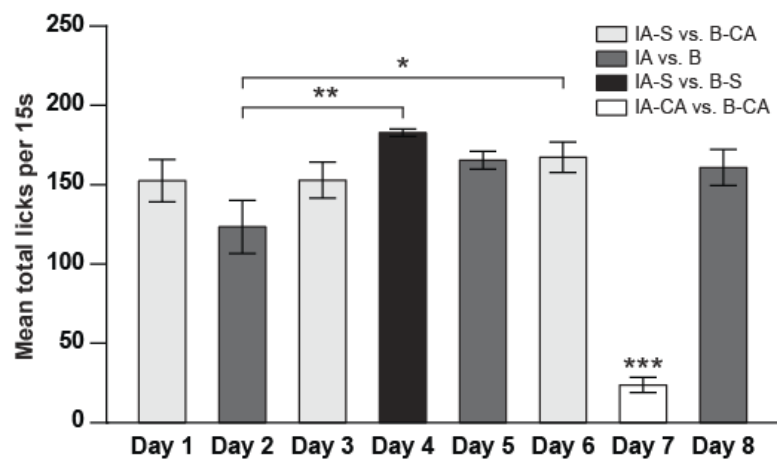


Figure 7. Mean total licks per 15s (\pm SEM) across days 1-8 of the congruency vs. hedonic value experiment. Compared to all other two-bottle brief-access choices, the mean number of total licks was significantly less on day 7 (0.01% isoamyl acetate-0.3 M citric acid (IA-CA) and 0.01% benzaldehyde-0.3 M citric acid (B-CA)) from all other trial days. Also, the mean number of total licks for day 2 (the first day of IA vs. B) were significantly different from those of day 4 (IA-S vs. B-S) and day 6 (IA-S vs. B-CA). * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

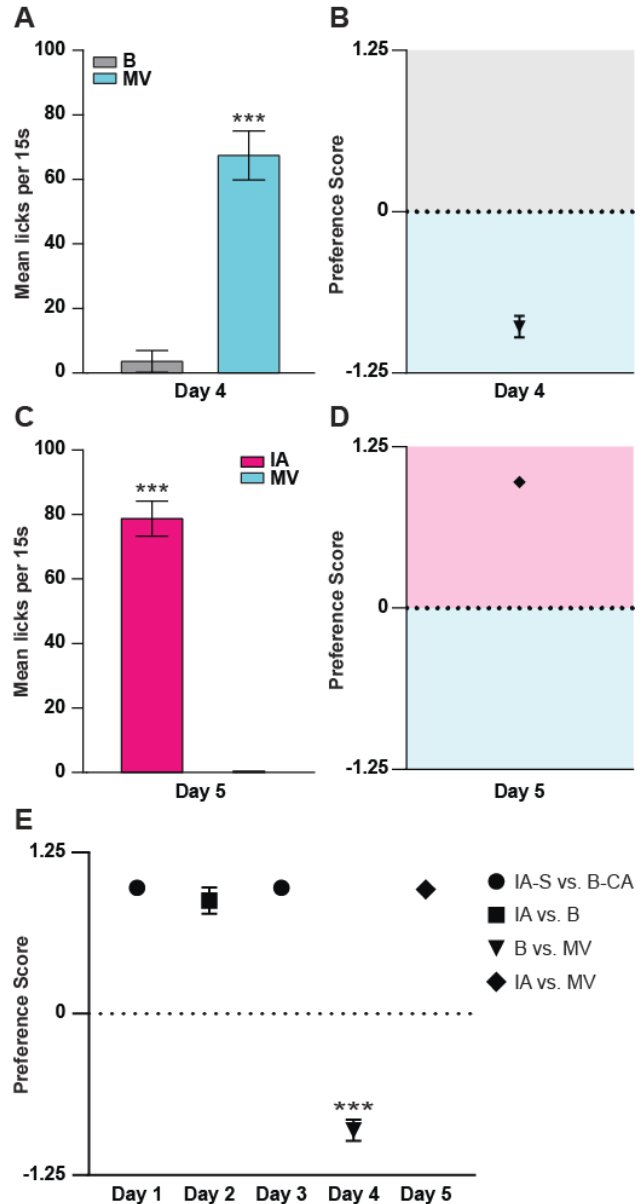
number of total licks for both bottles significantly differed across task days ($F(7, 49) = 26.80, P < 0.001$) (Figure 7). A *post hoc* analysis comparing the mean number of total licks for each task day revealed that rats sampled the bottles significantly less the first day they were given the choice between bottles containing odorized-water only (day 2: 126.62 ± 16.84) compared to the task day when both bottles contained sucrose (day 4: 186.51 ± 2.31 ; $t_{(7)} = 8.464, P < 0.001$) and the last day of isoamyl acetate-sucrose vs. benzaldehyde-citric acid (day 6: 170.83 ± 9.79 ; $t_{(7)} = 3.189, P < 0.05$). Most importantly, the mean number of total licks for bottles with congruent and incongruent odor-taste mixtures containing citric acid (day 7: 25.92 ± 4.82) was significantly less than all other task days (day 1: 156.01 ± 13.53 ; $t_{(7)} = 9.366, P < 0.001$; day 2: 126.62 ± 16.84 ; $t_{(7)} = 7.245, P < 0.001$; day 3: 156.33 ± 11.40 ; $t_{(7)} = 9.384, P < 0.001$; day 4: 186.51 ± 2.32 ; $t_{(7)} = 11.55, P < 0.001$; day 5: 169.00 ± 5.97 ; $t_{(7)} = 10.29, P < 0.001$; day 6: 170.93 ± 9.79 ; $t_{(7)} = 10.43, P < 0.001$; day 8: 164.55 ± 11.53 ; $t_{(7)} = 9.975, P < 0.001$). Taken together, these results show that rats prefer odor-taste mixtures containing an odor that was previously paired with a pleasant taste. However, when both odor-taste mixtures contain an unpleasant taste, rats sample both bottles significantly fewer times.

Experiment 2B: Odor Neophobia vs. Experienced Unpleasant Odor

While rats avoid stimuli containing a novel odor and stimuli containing an odor previously paired with an unpleasant taste, it remains unclear whether preferences for these unpleasant odor stimuli differ. Using the same animals as in Experiment 2A, I employed a two-bottle brief-access odor preference task to

Figure 8. Rats prefer a novel odor to an experienced-unpleasant odor, but prefer an experienced-pleasant odor. **(A)** Means licks per 15 s (\pm SEM) during the two-bottle brief-access task on day 4 of the unpleasant-experienced vs neophobia experiment. Rats given the choice between 0.01% benzaldehyde-odorized water and 0.01% methyl valerate, sample significantly more methyl valerate. **(B)** Preference score (\pm SEM) for 0.01% benzaldehyde-odorized water and 0.01% methyl valerate. A positive preference score indicates a preference for benzaldehyde and a negative preference score indicates a preference for methyl valerate. **(C)** Means licks per 15 s (\pm SEM) during the two-bottle brief-access task on day 5. Rats given the choice between 0.01% isoamyl acetate and 0.01% methyl valerate, sample significantly more isoamyl acetate. **(D)** Preference score (\pm SEM) for 0.01% isoamyl acetate and 0.01% methyl valerate. A positive preference score indicates a preference for isoamyl acetate while a negative preference score indicates a preference for methyl valerate. **(E)** Preference scores (\pm SEM) across all trial days 1-5. A positive preference score indicates a preference for 0.01% isoamyl acetate (Days 1,2,3,5) or 0.01% benzaldehyde (day 4 only). while a negative preference score indicates a preference for 0.01% benzaldehyde (days 1-3) or 0.01% methyl valerate (days 4 and 5). The preference score for day 4 was significantly different from all the two-bottle brief-access preference scores. *** $P < 0.001$.

determine whether rats prefer a



novel odor, methyl valerate, or a known unpleasant odor, benzaldehyde (Figure 8). First, I determined whether preferences for chemosensory stimuli differed from those in Experiment 2A. The results of a mixed-effects model analysis showed that preference ratios for the choice between isoamyl acetate-sucrose/benzaldehyde-citric acid did not differ from those in Experiment 2A [$F(4, 28) = 1.094$, $P = 0.3790$], nor did the preference ratios for isoamyl acetate/benzaldehyde [$F(3, 20) = 2.809$, $P = 0.0658$]. Having shown that chemosensory preferences remained unchanged,

I next sought to determine whether rats prefer to sample from a bottle containing the novel odor methyl valerate or a bottle containing the known, but unpleasant odor benzaldehyde (Figure 8A, B). Results from a two-tailed paired *t*-tests revealed that rats significantly preferred the novel methyl valerate to the known unpleasant benzaldehyde (MV: 68.63 ± 7.67 vs. B: 4.14 ± 3.43 ; $t_{(7)} = 7.064$, $P = 0.0002$). To determine if methyl valerate was inherently pleasant or confused for the experienced pleasant odor, a preference task between pleasant isoamyl acetate and methyl valerate was performed (Figure 8C, D). Results of a two-tailed paired *t*-tests showed that isoamyl acetate was significantly preferred to methyl valerate (IA: 80.04 ± 5.47 and MV: 0.53 ± 0.21 ; $t_{(7)} = 14.55$, $P < 0.001$). Results of a mixed-effects model analysis revealed a significant difference in the preference scores across all two-bottle choices ($F(4,27) = 231.2$, $P < 0.001$) (Figure 8E). A *post hoc* analysis revealed that the preference score for benzaldehyde/methyl valerate (-0.899 ± 0.0835) differed significantly from all other preference scores (day 1, IA-S vs. B-CA: 0.999 ± 0.0004 ; $t_{(27)} = 24.43$, $P < 0.001$; day 2, IA vs. B: 0.899 ± 0.1011 ; $t_{(27)} = 22.35$, $P < 0.001$; day 3, IA-S vs. B-CA: 0.999 ± 0.0004 ; $t_{(27)} = 24.43$, $P < 0.001$; day 5, IA vs. MV: 0.987 ± 0.0053 ; $t_{(27)} = 24.26$, $P < 0.001$). Taken together, the results of Experiment 2B show that odor-taste preferences are resistant to extinction, that rats prefer a novel odor to a known unpleasant odor, while preferring a known pleasant odor to an unpaired odor.

DISCUSSION

The results of this study provide evidence for how preferences for orally consumed odors and odor-taste mixtures are modulated by experience. Specifically, that an orally consumed odor is avoided until it is paired with a pleasant taste (Experiment 1), that odor-taste mixtures containing an odor that was previously paired with a pleasant taste, are preferred to congruent odor-taste mixtures (Experiment 2A), and odorized-water containing a novel odor is preferred to water odorized with one previously paired with an unpleasant taste (Experiment 2B). Taken together, these findings suggest that consummatory behaviors are guided by experience-dependent modulation of chemosensory processing.

Since most mammals are neophobic and avoid ingesting novel chemosensory stimuli, experience is essential for preference formation related to future food choices (Schifferstein and Verlegh, 1996; Amsellem and Ohla, 2016). Traditional experimental approaches to assess neophobia to novel chemosensory stimuli have employed a single bottle task (Miller and Holzman, 1981; Miller et al., 1986; Lin et al., 2009b, 2012). In this paradigm, animals have limited access to a single bottle containing a novel chemosensory stimulus. Over time, and without access to other liquids, animals sample the novel stimulus more and more. This task shows a reduction in avoidance of the stimulus as it becomes more familiar, but it does not assess whether the value of the stimulus has changed. For this reason, I decided to use a two-bottle brief-access preference task (see Methods).

Since both bottles are presented simultaneously for a limited period of time, rats can choose which stimulus it prefers to consume. Using the number of licks as a measure, this task assesses which of the two stimuli the animal prefers to sample.

Neophobia is a common mammalian trait (Barnett, 1958; Pliner and Salvy, 2006). Rats, in particular, display pronounced neophobia to orally consumed odors (Miller et al., 1986; Lin et al., 2009b). In Experiment 1, rats were given the choice between isoamyl acetate odorized-water and water. Unlike a single bottle task, where rats increase intake of odorized water over time, rats given a choice preferred water to odorized-water for multiple days (Figure 4A). Previous studies have shown that the affective value of an orally consumed odor is altered after being paired with a palatable taste (Fanselow and Birk, 1982; Stevenson et al., 1995; Prescott et al., 2004; Gautam and Verhagen, 2010; Green et al., 2012). In this study, after rats were given a single experimental session with isoamyl acetate paired with sucrose (IA-S), the preference for water over isoamyl acetate odorized-water was eliminated (Figure 4B). Taken together, these results suggest that novel odorized-water is avoided due to neophobia, and when given a choice, rats continue to avoid it. Only after experience with an odor-taste mixture containing a pleasant taste does their preference for water and odorized-water shift.

The results of Experiment 1 show that a single experience with an odor-taste mixture can change odor preferences. Sampling an odor mixed with a taste generates robust odor-taste associations, where the odor acquires the quality and hedonic value (pleasantness/unpleasantness) of the taste (Fanselow and Birk,

1982; Schul et al., 1996; Sakai and Yamamoto, 2001; Sakai and Imada, 2003). To examine how previous experience with a pleasant and an unpleasant odor-taste mixture influences chemosensory preferences, Experiment 2A sought to determine whether the value of an experienced odor or the congruence between the odor and taste was more influential in guiding preferences for odor-taste mixtures. First, water regulated rats (see Methods) were given three days of home cage experience with two odor-taste mixtures, one pleasant 0.01% isoamyl acetate-0.2 M sucrose and one unpleasant 0.01% benzaldehyde-0.3 M citric acid (IA-S vs. B-CA). Although previous studies have shown that three days of experience with odor-taste mixtures results in robust odor-taste associations, which are resistant to interference and extinction (Sakai and Yamamoto, 2001), sampling incongruent odor-taste mixtures (day 4 and day 7) could have changed established chemosensory preferences. Therefore, the day following an incongruent odor-taste mixture session, preferences were determined for odorized-water mixtures (IA vs. B), followed the next day by the choice between the congruent odor-taste mixtures (IA-S vs. B-CA). These results showed that rats consistently preferred stimuli containing isoamyl acetate regardless of sampling incongruent odor-taste mixtures (Figure 5).

Although the preference scores did not significantly differ between the two-bottle choices (Figure 6E), the mean number of total licks (i.e. how many times the rats sampled from either bottle) was significantly less when both bottles contained citric acid (day 7). This is an important distinction between the preference score and the mean number of total licks. The preference score is a measure of which

stimulus is preferred, regardless of how much they are sampled, whereas the mean number of total licks is a measure of the motivation to consume either stimuli relative to the other two-bottle choices. When both odor-taste mixtures contained citric acid, rats preferred to drink from the bottle containing isoamyl acetate; however, they were not willing to continually drink from the bottles due to the aversiveness of citric acid. Furthermore, the mean number of total licks for the first day rats were given the choice between odorized-water (day 2, IA vs. B) was significantly less than the mean number of total licks when both odor-taste mixtures contained sucrose (day 4, IA-S vs. B-S) and the last day of congruent mixtures (day 6, IA-S vs. B-CA). I hypothesize that the decrease in motivation is related to the novelty of the task. Up until this two-bottle choice, rats had always been presented with odor-taste mixtures. Day 2 was the first time rats had ever experienced only odorized-water. I speculate that if rats had been given home cage experience with odorized-water, as they had with odor-taste mixtures, the mean number of total licks would not have been significantly different. This hypothesis will be tested in future experiments.

Preferences for odor-taste mixtures can be influenced by other factors as well, such as the solution's caloric value. As reviewed by Sclafani 2001, a non-caloric taste solution paired with an intragastric infusion of calorically-rich carbohydrate is preferred to a non-caloric taste solution paired with intragastric infusion of water. In my experiments, only the sucrose-containing solutions had a caloric value, making them more positive than a citric acid solution, which had no caloric value. It is possible that the absence of caloric value, in combination with

the high concentration of citric acid, makes the benzaldehyde-citric acid solutions even more disgusting and less preferred. By pairing isoamyl acetate with sucrose, isoamyl acetate takes on the positive taste and postingestive effects of sucrose. Regardless, the effect of pairing isoamyl acetate with sucrose is hedonically positive and accounts for the preference for IA-CA solutions over B-CA solutions on day 7 of Experiment 2A.

Taken together, these results show that the hedonic value of an odor previously paired with a taste is more influential than the 'correctness' of an odor-taste mixture (i.e., congruence vs. incongruence). These findings are consistent with human psychophysical experiments showing that previous experience with flavors modulates the perceptual qualities of congruent and incongruent odor-taste mixtures (Schifferstein and Verlegh, 1996; Small et al., 2004; Labbe et al., 2006; White and Prescott, 2007; Shepard et al., 2015).

The results of Experiment 1 and Experiment 2A, showed that both a novel odor and an odor previously paired with an unpleasant taste are avoided. However, it was unclear whether an experienced-unpleasant odor or a novel odor would have a greater influence on chemosensory preference. Since experienced odor-taste associations are resistant to extinction (Best et al., 1978; Sakai and Yamamoto, 2001), the same group of rats as Experiment 2A were given a single day of reintroduction to the initial hedonic odor-taste pairings (IA-S vs. B-CA). On day 4 of Experiment 2B, rats were given a choice between benzaldehyde (experienced-unpleasant odor) and methyl valerate (novel odor). Surprisingly, rats showed a strong preference for methyl valerate over benzaldehyde (Figure 8A, B).

To ensure that methyl valerate was not inherently positive or rats were confusing it with isoamyl acetate, rats were given the choice between methyl valerate and isoamyl acetate (day 5). Rats drank significantly more isoamyl acetate, demonstrating that the two odors were perceptually different (Figure 8C, D). These results suggest that the experienced-unpleasant odor is more aversive than a novel odor. However, the rats in this task were highly trained and, to my knowledge, had never become ill after sampling chemosensory stimuli in the two-bottle brief-access task. It is possible that the consistency of the task afforded the rats the assumption that a novel chemosensory stimulus was not a threat. Future studies will examine whether a more unpredictable task (i.e. taste-potentiated odor aversion) will alter preferences between an experienced-unpleasant and novel odor stimulus.

In conclusion, the results of these experiments show that chemosensory preference is highly driven by the hedonic value of an odor. Once neophobia is overcome and rats sample a novel odor, they show a strong preference for water until the odor is paired with a taste. When the odor is paired with a pleasant taste, the odor is associated with the hedonic value of the taste. After an initial odor-taste association is made and a preference is formed, preference for future odors and odor-taste pairings will remain consistent with the hedonic value of the odor. As shown in the congruency vs. hedonic value experiment (Experiment 2A), rats will continually show a preference for a chemosensory stimulus containing a hedonically pleasant odor. When presented with the choice between an experienced-unpleasant odor and a novel odor, rats will surprisingly prefer the

novel odor under the conditions stated in the unpleasant-experienced vs. neophobia experiment (Experiment 2B). This shift in preference could indicate that rats prefer a novel stimulus to something that is known to be unpleasant; had the task conditions been different (i.e. conditioned taste aversion or taste-potentiated odor aversion), rats may have been more hesitant to sample the novel stimulus. Future research will investigate how prior aversive experiences modulate preferences for novel and experienced chemosensory stimuli.

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Kelsey A McQueen

EDUCATION

2013 B.S., Biology (concentration: cellular physiology), minor: French language,
University of Louisville.

Original Reports

Conference abstracts and proceedings

- ## CLINICAL/MEDICAL EXPERIENCE

39

- Testing gustation/olfaction ability of anosmic rats via 0.125% Triton X 100 nasal inactivation
- Confocal Imaging of taste buds using whole-cell mount technique
- Cresyl Violet Staining and mounting of whole rat brain coronal sections

Undergraduate Independent Research

July 2015-May 2017

- Conducted experiments to identify levels of AdipoR1 receptors in cardiac tissue of rats ranging from 3-9weeks of age
- Effects of varying molecular forms of adiponectin on development
- Effects of puberty on adiponectin circulation and production of adiponectin by cardiac muscle tissue

Phi Delta Epsilon Pre-Med Fraternity

January 2014-January 2017

- Secretary Chair
- Involvement in medical simulations conducted by medical officers of the US Army
- Volunteered for Supplies Overseas
- Fundraising for Norton Children's Hospital

HOSPARUS

November 2015-August 2017

- In-home and nursing home patient care

Medical Scribe

March 2016-June 2016

- Norton Hospital Emergency Department

University Hospital Emergency Room

August 2015-August 2017

- volunteer in maintenance and stocking facilities

Surgical Observations to Neurosurgeon

November 19, 2013; December 11, 2013

- Dr. Altstadt with University of Louisville Hospital in Louisville, KY
- Shadower during clinical hours visiting post and pre-op patients
- Observed a C4-7 Posterior cervical laminectomy, duraplasty syringosubarachnoid shunt, evacuation of acute subdural hematoma

Observation of Pediatric Physician May 13, 2014

- Dr. Bolling with Pediatric Associates in Ft. Thomas, KY
- Observed with 10 patients in annual examinations

Surgical Observation to Colorectal Surgeon April 22 and 29, 2014

- Dr. Katz with University of Louisville Medical School Admissions and Jewish Hospital in Louisville, KY
- Scrubbed-in on colonoscopy and malignant pullup extractions

Surgical Observation to Neurosurgeon July 22, 2014

- Dr. Bailey with Christ Hospital Spine Surgery Center in Cincinnati, OH
- Observed a ACDF Spine Stimulator trial, spine stimulator adjustment, and laminectomy

INTERNATIONAL/DOMESTIC VOLUNTEER AND ACADEMIC WORK

Youth Group Leader September 2017-present

- First Christian Church of Louisville
- Lead activities and facilitate personal and spiritual conversations with youth group participants
- Lead service trips

Mission Trip June 24-30 2018

- Hamilton, OH
- Deck demolition and repair, shed demolition, repair, and painting

Study Abroad June 24 - July 24 2016

- French Riviera
- Studied French Language at Centre International d'Antibes

Service Trip to Guatemala June 1-7 2013

- Worked and lived in Antigua
- Built cement-block stoves in village homes to ensure safety

SKILLS

Fluency in French language

Expertise in laboratory research equipment/method

Proficiency with Epic System

Mastery of Microsoft Word, Excel, and Powerpoint

ADDITIONAL EMPLOYMENT

Lifeguard for the University of Louisville	September 2017-May 2018
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Team Lead Lifeguard for Kentucky Kingdom	May 2015-October 2017
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University of Louisville Student Recreation Center	May 2015-May 2017
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Lifeguard for the Diocesan Catholic Children's Home	May 2011-August 2014
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